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PRINCIPAL INVESTIGATOR: Yvedt L. Matory, M.D.

CONTRACTING ORGANIZATION: Brigham and Women's Hospital
Boston, Massachusetts 02115

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Yvedt L. Matory, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Brigham and Women's Hospital
Boston, Massachusetts 02115

E-Mail: ymatory@partners.org

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Final Report for Award Number DAMD17-00-1-0608

Yvedt L. Matory, M.D.

Department of Surgery

Brigham and Women's Hospital

Specific Aim I To evaluate the role of mammary cell volume reduction in inducing morphologic changes in residual tissue. Muller (9) observed that the mammary tissue in these mice is initially hyperplastic and eventually becomes cancerous. Therefore any hyperplastic tissue remaining after mastectomy is at risk for developing breast cancer. In these experiments, we assessed the extent of that risk by evaluating the volume and morphology of mammary tissue remaining after PM.

We evaluated the volume of residual mammary tissue after mastectomy by using autopsy studies performed one week after PM . This time interval was chosen to allow full recovery from surgery and it precedes the development of scar tissue that would interfere with pathological evaluation. The skin was cut in .25 inch serial sections and stained with hematoxlyn and eosin. The specimens will then be examined for the presence of mammary tissue. This required approximately 20 animals. Note; the original proposal called for the use of anti-MMTV antibody to screen for the presence of residual mammary gland tissue. Unfortunately, the quantity of antibody obtained was too small to screen a large number of mice. As a result only H&E staining was used for these experiments.

Findings:

Mammary gland histology - Histologic findings confirmed that 10-12 mammary glands were removed from each animal. Using the techniques described above, residual mammary gland tissue was found in 5% of animals after mastectomy .

B. We evaluated the influence of reducing the volume of mammary tissue on the development of mammary cancer by performing partial mastectomies. One quarter and one half of the breast tissue was excised, using techniques previously described (13). The animals were observed for the development of tumor.

All procedures were performed on 3 month old animals. In these mice, mating accelerates the development of breast cancer. Control (20 animals) and experimental animals (20 each group) were then mated. Animals were sacrificed at varying intervals (50, 70, and 90 days- 120 animals) after mating in

order to capture mammary glands at different stages of malignant transformation. For all experiments, the animals were sacrificed when any single tumor they developed reached a size of more than 2.5 cm in any single dimension.

Findings:

Tumor occurrence after partial excision- We performed six separate experiments designed to evaluate the effect of prophylactic mastectomy in this model. The mean age of the animals was 3.5 months. The experiments contained five to ten animals in each group. All control animals developed tumor. The mean day of onset of tumor was 54 +/- 16 days after the experiment was initiated. In animals that underwent prophylactic mastectomy, only 41% developed tumor by 120 days. In animals that developed tumor, the onset of tumor was later than that of control animals with a mean of 103 +/-20 days. Partial excision of mammary glands was equivalent in tumor onset to that of control animals.

Specific Aim II To evaluate the effect of mammary cell volume reduction on transgene expression. These studies were performed to determine if mastectomy has any influence on transgene expression in residual breast tissue. Muller (9) reported a correlation between transgene expression and morphologic changes that progress from hyperplasia to cancer in mammary tissue. The following experiments were done to determine if there are transgene changes subsequent to mastectomy. The procedures were performed on 3 month old mice. Control animals were mated but did not have any type of mastectomy (60 animals). Transgene expression was tested using PCR analysis of tumors that grow in operated animals.

Findings:

The stability of Her2 neu expression was evaluated in three separate experiments. 1)Five mice were tested monthly for six months. Gene expression was stable for the duration of this period. 2) Mice at different ages, 1 month old, 2 months old up to six months old, were also evaluated for c-nue expression. Again expression was stable for the period of study. 3) Mice were tested before and after prophylactic mastectomy for transgene expression. There were no detectable changes in gene expression.

A. Testing for the MMTV/nue* oncogene was correlated with the degree of malignant transformation of mammary tissue. These experiments required 20 animals in each group (full, quarter

and half mastectomy-60 animals) and were performed on 3 month old mice. Control and experimental animals were mated. Animals were sacrificed at various intervals (50,70, and 90 days-180 animals total) after mating in order to capture mammary glands at various stages of malignant transformation.

Findings: C-nue expression was stable for the period of study as a result there was no correlation with tissue differentiation.

Specific Aim III Evaluate the Effects of Troglitazone in the model with and without PM

There is evidence that this agent has antitumor effects on genetic cancers. Its effects are mediated by the PPAR-g nuclear receptor pathway. Studies were done to determine if the effects of PM are enhanced or replaced. The experiments described in Specific Aim II were repeated. Control animals received no intervention and experimental animals received Troglitazone. Troglitazone treatment (200 mg/kg, Gavage, 50 days) started 2 days after surgery and continued for a month. To determine if the antitumor effects are mediated by the PPAR-g nuclear receptor pathway, animals were tested for PPAR-g expression before and after mating and before and after the administration of Troglitazone.

A. Full prophylactic mastectomies were performed. Control animals were mated (no mastectomy) and received Troglitazone(60 animals).

Findings: We performed three experiments in which animals received Troglitazone alone (TG) and Troglitazone in combination with PM (TG/PM). Control animals received no intervention or PM. At 117 days 11/23 TG animals developed tumor mean onset 75 days+/- 30, all control animals developed tumor by 85 days and animals with TG/PM at 120 days only 4/12 had tumor. These results demonstrate some promise , but they are preliminary.

B. Testing for the MMTV/neu* oncogene was correlated with the degree of malignant transformation of mammary tissue.

Findings: C-nue expression was stable for the period of study as a result there was no correlation with tissue differentiation.

Summary: PM appears to be effective in reducing the incidence ad in delaying the onset of tumor in genetically predisposed mice. Troglitazone appears to enhance these effects. These findings cannot be explained by alterations in MMTV/neu transgene expression.

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